Revision 1

Store at -20C

#28692

5-Methylcytosine (5-mC) (D3S2Z) Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	Source/Isotype:
IF-IC, Dot Blot	All	Endogenous	Rabbit IgG

Product Usage Information

Application	Dilution
Immunofluorescence (Immunocytochemistry)	1:1600
DNA Dot Blot	1:1000

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity/Sensitivity

5-Methylcytosine (5-mC) (D3S2Z) Rabbit mAb recognizes endogenous levels of 5-methylcytosine. This antibody has been validated using ELISA, dot blot, and MeDIP assays and shows high specificity for 5-methylcytosine.

Source / Purification

Monoclonal antibody is produced by immunizing animals with 5-methylcytidine.

Background

Methylation of DNA at cytosine residues is a heritable, epigenetic modification that is critical for proper regulation of gene expression, genomic imprinting, and mammalian development (1,2). 5-methylcytosine is a repressive epigenetic mark established *de novo* by two enzymes, DNMT3a and DNMT3b, and is maintained by DNMT1 (3, 4). 5-methylcytosine was originally thought to be passively depleted during DNA replication. However, subsequent studies have shown that Ten-Eleven Translocation (TET) proteins TET1, TET2, and TET3 can catalyze the oxidation of methylated cytosine to 5-hydroxymethylcytosine (5-hmC) (5). Additionally, TET proteins can further oxidize 5-hmC to form 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC), both of which are excised by thymine-DNA glycosylase (TDG), effectively linking cytosine oxidation to the base excision repair pathway and supporting active cytosine demethylation (6,7).
Normally DNA methylation occurs in a bimodal fashion, such that CpG dinucleotides are largely methylated across the genome, except in short stretches of CpG-rich sequences associated with gene promoters, known as CpG-islands, where methylation is virtually absent (8). Cancer cell genomes often undergo global hypomethylation, while CpG-islands become hypermethylated, causing their associated promoters to become repressed (9). There is evidence that a number of aberrantly hypermethylated CpG-islands found in carcinomas occur at tumor suppressor genes such as RB1, MLH1, and BRCA1 (10).

Background References

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3. Okano, M. et al. (1999) *Cell* 99, 247-57.
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5. Tahiliani, M. et al. (2009) *Science* 324, 930-5.
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7. Ito, S. et al. (2011) *Science* 333, 1300-3.
8. Suzuki, M.M. and Bird, A. (2008) *Nat Rev Genet* 9, 465-76.
9. Berman, B.P. et al. (2012) *Nat Genet* 44, 40-6.
10. Sproul, D. and Meehan, R.R. (2013) *Brief Funct Genomics* 12, 174-90.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Applications Key

IF-IC: Immunofluorescence (Immunocytochemistry) **Dot Blot:** DNA Dot Blot

Cross-Reactivity Key

All: All Species Expected

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